“BIOPROSPECTING OF ANTIDERMATOPHYTIC, HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF *BALIOSPERMUM* SPECIES ”

A

Synopsis submitted to

Swami Ramanand Teerth Marathwada university,Nanded.

For the degree of

Doctor of philosophy in Botany

Under the faculty of science

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March- 2022.

"BIOPROSPECTING OF ANTIDERMATOPHYTIC, HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF BALIOSPERMUM SPECIES "

1. RESUME OF PROPOSED WORK OR PROBLEM

Dermatophytes are filamentous fungi that survive on keratinous materials. There is broad prevalence of dermatophytes infection among the world. Dermatophytosis is the disease that mainly caused by different species of dermatophytes within the cutaneous layer of the skin.

Various parts of the *Baliospermum* species are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments.

The present study revealed some notable pharmacological activities of the *Baliospermum* species antidermatophytic, antioxidant, hepatoprotective and cytotoxicity assay of *Baliospermum* species from Marathwada.

2. INTRODUCTION:

Dertmatophytosis is the most common cause of dermal fungal infection in throughout the world. In the recent past, there has been alarming rise in patients presenting with atypical types of dermatophytosis. These patients require prolonged treatment with systemic and topical antifungal agents. Frequent relapses and extensive disease affect the quality of life of the patient significantly. These types of infections are mainly caused by host factors such as comorbidity and immunosuppression; hot and humid climate, life style changes and poor hygiene are responsible for atypical dermatophytosis. There are several atypical clinical types ofdermatophytosis reported in india such as psoriasis-like, eczematous dermatitis-like, seborrhic dermatitis like and rosacea**(Sunil Dorga and TarunNarang,2017).**

Dermatophytosis is a superficial fungal infection with an affinity for keratin-rich structures such as skin, hair and nails. This disease is predominantly caused by Arthrodermataceace family that contains 40 species divided among three different genera, i.e. *Trichophyton, Epidermophyton,* and *Microsporum.*The nature of predominant species of fungus

causing dermatophytosis differs from region to region. An epidemiological transformation of the disease has been observed over last 5-6 years **(Singh et.al., 2020).**

Dermatophytosis or tinea is predominance in about 20%-25% of all total world populations. Dermatophytes are filamentous fungi naturally living on keratinous materials found in soil. Dermatophytosis or tinea mainly caused by dermatophytes. Dermatophytosis or tinea can be found on the skin of different parts of the human body which make it takes various names based on the infected area such as tinea pedis on the feet, tinea unguium on the nails, tinea capitis on scalp,tinea cruris on the groin, and tinea corporis on the body.

It is considered a prevalent skin disease worldwide. Moisture and warm conditions are the most suitable factors for a wide distribution of dermatophytosis in tropical countries. This epidemiological distribution may change with migration, life style, immunosuppressive state, drug therapy, and socioeconomic conditions. The treatments of dermatophytosis needs about 2-4 weeks to be cured in many types and may require many months in case of tineacapitis and onychomycosis**(Al-Khikani2020).**

In the past decade, the incidence of dermatophytosis has risen dramatically. The humid weather, over population and poor hygienic conditions are ideally suited for the growth of dermatophytes and these factors are more important in a tropical country like India. Among the infectious diseases, diseases caused by fungal infections account for a larger proportion of health problems in humans, particularly among women and children**(Vaijayanthimalaet.al., 2004).**

Mycotic infection of the scalp, tineacapitis, is common disease in developing countries. The use of medicinal herbs in the treatment of skin diseases including mycoticinfection is an age-old practice in many parts of the world. This use has been supported by the isolation of active antifungal compounds from the plant extracts. These compounds represent secondary metabolites that serves as defense agents against invading micro-organisms **(Ali-Shtayeh and Abu Ghdeib, 1998).**

According to world health organization (WHO) 80% of population in developing countries depend on medicinal plants for their primary health care. India is one of the hotspots of

biodiversity for medicinal aromatic plants in the world. Medicinal plants produces large amount of secondary metabolites which are helpful for human health care and maintain resistance against infection. A number of medicinal plants showed effective antimicrobial activities which will be comparable to synthetic standard drugs**(Raveesha and Sushma, 2017).**

Baliospermum montanum (Mueell - Arg) is an aromatic medicinal plant belonging to the family Euphorbiaceae includes 280 genera with 730 species with the largest genus Euphorbia. Root, leaf, and seeds of *Baliospermu mmontanum* are used medicinaly and are documented from asian countries, including Nepal, Burma, Malaya, and India. *B. montanum* is known for its ethnobotanical and traditional use **(Radhakrishna and Kumari, 2019).**

Almost all the parts of danti are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as purgative , anthelmintic, diuretic, diaphoretic, rubefacient, febrifuge and tonic. They are also reported to be useful in dropsy, constipation, jaundice, leprosy, and skin diseases **(Mali and Wadekar,2008).**

The use of plants or their primary and secondary metabolites for curing human and/ animal diseases has long being in existence in India from the ancient times. Liver injure treatments are among the most important of today's research domains, because with every passing year there is a more acute need for liver transplants. At modern time human beings have take n many allopathic drug, toxic influence and food hazards which lead to some form of liver disorders. That is why today's attention is drawn to the potentials of few miracle plants that have ability to reduce or cure liver damage**(Savita Rani et.al. 2014).**

In Ayurveda, plant ingredients have been used to protect against liver injury through various chemicals and dietary ingredients. Therefore, herbal medicines are safe and have the potential to cure such diseases, so they have gained popularity in recent years. Long-term use of these drugs is very beneficial. Many medicinal plants present in different parts of India have been referred to as hepatoprotective drugs and are widely used to treat liver disorders. Various plant and polyherbal formulations have hepatoprotective action (Raj et al.2009). About 160 phytoconstrictors and other phytochemicals have been claimed to have hepatoprotective activity. More than 87 plants are used in India, of which 33 have patents and have proprietary multi-component plant formulations (UNIDO, 1996). Ayurveda, the ancient system of Indian

medicine, mentions various medicinal plants for the management of liver disorders. Many herbal formulations like Liv-52 and *Ocimum sanctum* Linn. (Labiatae) are available to treat liver disorders. Similarly, flavonoids are phenolic compounds that are widely distributed in plants and have many biological effects, including antioxidant and free radical cleansing abilities ((Goel and Dhawan, 1991; Mitra et al., 1998; Chattopadhyay et al., 1992; Middleton et al., 2000).

In addition, man uses many synthetic drugs in the disease conditions, which are foreign to the organs of the body and can produce various toxic manifestations. The management of liver disease is still a challenge facing modern medicine. It is therefore necessary to find alternative drugs for the treatment of liver diseases that will replace the current use of drugs with dubious efficacy and safety (Rajesh and Latha, 2004).

Therefore, *Baliospermum* species from Marathwada are selected for the present study. A survey of the literature reveals that practically no more work has been done on Antidermatophytic,Hepatoprotective, Antioxidant and Cytotoxicity Assay of *Baliospermum*Species. The present research work is, therefore, undertaken for the study.

3.REVIEW OF THE LITERATURE:

Radhakrishna and Kumari (2019) have testedantifugal activity by plate diffusion method against three pathogens,*Aspergilusniger*, *Aspergilusflavus* and *Candida albicans.* A clear zone of inhibition observed around the wells confirming of plant *B. montanum* by the terpenoid fraction. Candida albicans showed the highest degreeof inhibition when compared to *Aspergilusniger* and*Aspergilusflavus*. This study clearly shows the potential of the terpenoid extract in the treatment of diseases caused by pathogenic fungi.

Raveesha and Sushma (2018) have screened aqueous methanol and chloroform extracts of root, stem, leaf and callus of *B. montanum* for potential antibacterial activity against selected bacterial strains by agar well diffusion method. Antifungal activity will be carried out by food poison technique against *Fusarium oxysporum*.Methanolic extract of leaf and callus showed maximum zone of inhibition against *Staphylococcus aureus*. Whereas, aqueous extract of stem, root exhibited maximum zone of inhibition against *Bacillus subtilis.*Methanolic extracts showed higher antifungal activity as compared to other extracts**.**

Bijekar et.al (2015) studied the methanolic leaf extract of *B. montanum* will be cast for GC-MS analysis. Theidentified compound may have medicinally value and possess various pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop safe drug.

Rout et.al (2017) have evaluated Danti *(Baliospermum montanum*) and their parts are used as an ingredient in 132 formulations and indicated in almost 28 disease conditions. Root has been used in maximum (125) formulations as an official part. Recent evidence based studies validates some of the classical therapeutic claims of external application of danti.

Arunporn et.al (2019) found the ethanolic extract of *Baliosprmum montanum* root can be a potential source of anti-allergy, anti-inflammation and anti-cancer compounds**.**

Vaijayanthimalaet.al (2004) evaluated turmeric oil and will be found to inhibit dermatophytes in vivo. *Cassia alata* shows the inhibitory activity against the dermatophytes using ethanol extracts. The ethanol extract of the whole plant of *Lawsonia inermis*showed antifungal activity against *Trichophyton mentagrophytes, Candida albicans, Candida neoformans, Aspergillus niger* and *Microsporum canis*.The essential oil of *Ocimum spp*. has been reported to have antimicrobial and antidermatophytic properties. *Psoralea corylifolia* seed essential oil showed moderate antifungal activity.Anti-dermatic activities of 23 South Indian medicinal plant materials have been studied using five isolates of *Trichophyton rubrum* and four isolates of *Trichophyton mentagrophytes*. Ethanol extracts of plants shows more activity than water extracts**.**

GC-MS will be carried out by using an Agilant Technology-7890 system coupled with Agilant 7000 GC-MS triplequaid and Agilant 7693 auto sampler equipped with a capillary column (30mmXo.25mm). Helium will be used as the carrier gas at a flow rate of 1ml/min. The oven temperaturewill be initially held at 70°C for 1minute and increased to 180° C at the rate of 30°C /min. finally the temperature will be increased to 280°C for 30 minutes. Interpretation of mass spectrum GC-MS will be done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component will be compared with the spectrum of the component stored in the NIST library and the name,

molecular weight and structure of the components of the test materials will be ascertained by **Radhakrishna and Kumari(2019).**

Throughout the history of mankind many infectious diseases have been known to be treated with herbal products. Natural products provide enormous opportunities for the development of new drugs, especially antibiotics, which may have the potential to treat infectious diseases. There is a constant need to find new antidermatophyticcompounds with the right chemical composition and new methods of action against antidermatophytic fungal pathogen.

Therefore, *Baliospermum* species from Marathwada are selected for the present study. A survey of the literature reveals that practically no more work has been done on evaluation of Antidermatophytic,Hepatoprotective, Antioxidant and Cytotoxicity Assay of *Baliospermum* Species. The present research work will be, therefore, undertaken with the following objectives:-

4.OBJECTIVES OF THE STUDY:

The objectives of the proposed work are as follows

- Collection, identification, authentification and phytochemical characterization of *Baliopermum* species.
- To extract the samples in different solvents for partitioning the plant ingredients of polar and non-polar nature soxhlet extraction and maceration method.
- To study phytochemical analysis of *Baliospermum* Species from Marathwada.
- To study the *in-vitro* anti-dermatophytic activity of selected plant extracts by disc diffusion and poisoned food assay.
- Determination of Minimum Inhibitory Concentration (MIC) of *Baliospermum* Species showing promising *in-vitro* anti-dermatophytic activity.
- To study *in-vitro*cytotoxicity and antioxidant activity of plant extracts.
- To study the *in-vivo* hepatoprotective potential of selected plant extracts by using suitable animal model.
- Statistical study of all approaches.

5.MATERIALS AND METHODS

5.1. Plant Material and Extract Preparation

5.1.1. Collection of Plant Material

The native medicinal plant (botanicals) *Baliospermum* species will be selected and collected from Marathwada region to evaluate their antidermatophytic,Hepatoprotective, Antioxidant and Cytotoxicity efficacy.

5.2. Cleaning and sterilization

5.2.1. Surface Sterilization of Plant Material

The collected plant material will be thoroughly will behed under the running tap water followed by surface sterilization with 1% Ethanol/H_2O_2 and then will be will behed with autoclaved distilled water. The surface sterilized plant material will then dried in oven at 40^0 C for 5 days or until they will be dried completely.

5.2.2 Cleaning and sterilization of glasswares and media

All the glass wares required for research work will be kept in cleaning solution for overnight. Cleaning solution will be prepared by dissolving 60g of potassium dichromate ($K_2Cr_2O_7$), 60ml of concentrated sulphuric acid (Conc. H_2SO_4) in one liter of water. Each of these chemicals will be dissolved separately in 500ml of water and finally mixed. All the materials to be used will be will be held thoroughly with sterile distilled water and then dried in hot air oven at 160^0C for 60 min.

The cleaned and dried petriplates will be wrapped with paper and sterilized in autoclave. Test tubes and flasks having culture media will be plugged with nonabsorbent cotton. These are covered with paper and further autoclaved at 121^0C at 15 PSI for 20min. The nichrome wire loop, blades, scalpel, forceps will also be dipped in ethanol and then sterilized by heating on the flame.

5.3. Test microorganisms and inoculum preparation.

All *Trichophyton rubrum, T. mentagrophytes, T. verrucosum, T. violaceum, T.schoenleinii, T. tonsurans, T. concentricum, T. equinum, Microsporum cannis, M. gypseum, M. audouinii, M. cookie, M. equinum, M.ferrugineum, M. gallinae, M. nanum,* and *Epidermophyton floccosum* reference strains of fungi will be used in the study will obtained from Microbial Type Culture Collection (MTCC) of Institute of Microbial Technology (IMTECH), Chandigarh (India). All these cultures will be preserved in the glycerol stock at − 80 °C. The inoculum of

these organisms will be prepared by 4 h activation in a suitable broth, followed by its standardization upto 0.5 McFarland standards.

5.4. Organic extracts preparation and their antifungal screening.

Different organic solvents such as water, ethanol chloroform and hexane will be screened to work out the best extractant. The organic extracts will be prepared by using solvent-solvent extraction of the aqueous extract. The aqueous extract will be prepared by suspending powdered plant material (17.5 g) in sterile distilled water (100 mL) and keeping it in hot water bath at 40 °C for 20 min. Hundred milliliter of filtered aqueous extract will be then shaken vigorously in a separating funnel with an equal volume of a particular organic solvent, for three independent times. The pooled organic layers will be concentrated in a rotavapor at 45 °C (under vacuum). The leftover material thus obtained will be dissolved in diluted Dimethyl Sulfoxide (DMSO) [(30% (v/v)], which will then be tested for their antimicrobial activity against the reference strains using following technique.

5.4.1. Disc Diffusion Assay

5.4.2. Evaluation of Antifungal Efficacy of Plant Extracts on Mycelial Growth of Fungal Pathogen through Poisoned Food Technique

5.4.3. Minimum inhibitory concentration (MIC) studies

The test preparations (ethanol extract, ethyl acetate extract, flavonoids, cardiac glycosides) will be studied for their MIC against the reference microbial strains (sensitive to the respective preparations in Agar Well Diffusion) by Agar dilution method, as per the protocol followed in Sood et al., (2020) using various concentrations prepared from their respective stock solutions. Hundred microliters (100 µL) of the activated test organism (adjusted to 0.5 McFarland standards) will be swabbed onto the solidified plates (containing different concentrations of a test preparation) and incubated at 25 °C for 18–24 h. The lowest concentration of the test preparations which inhibited the microbial growth will be taken as their MIC. The values will be compared with that of the standard antibiotics.

5.5. Qualitative and quantitative phytochemical analysis

5.5.1. Phytochemical Tests

Fresh stock solution of each extract will be prepared for each experiment. To prepare 100 mg/ml extract, 1g of either of the extract will be dissolved in 10 ml of distilled water, shaken vigorously to obtain a homogenous solution except for the glycoside test, for which 100 mg extract will be directly used. The procedures will be used earlierly described by Harborne (1973) and Sofowara (1993).

5.6 IN VITRO ANTIOXIDANT ACTIVITY

5.6.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

Free radical scavenging activity of *Baliospermum* species will be determined by using DPPH assay (Wang et al. 2008). The reaction mixture containing a mild series (10–100 gm/ml) of plant phytochemicals will be incubated in MeOH (3.0 ml; 0.3 mM) with DPPH solution. The ingredients will be mixed vigorously and allowed to stand for 30 minutes at room temperature. The absorbance will be measured at 517 nm. The lower absorbance indicates high DPPH scavenging activity. Ascorbic acid will be used as the reference antioxidant. The percent inhibition of DPPH will be calculated using the formula, [(C–T/C)] x 100, where C is absorbance of the control and T for the test samples.

5.6.2 Hydroxyl Radical Assay

The hydroxyl radical scavenging activity of *Baliospermum* species will be determined by Yang and Guo, 2001. The reaction mixture containing dilution series from 10 to 100 lg/ml of *Baliospermum* species will be incubated with deoxyribose (3.75 mM), H_2O_{23} (1 mM), $FeCl_3$ (100 lM) in phosphate buffer (pH 7.4). The reaction is terminated by thiobarbituric acid (1 ml; 1 % w/v) and trichloroacetic acid (1 ml; 2 % w/v) and then heated in boiling water bath for 15 min. The pink chromogen formed eventually resulted in the formation of thiobarbituric acid reactive substances (TBARS). The content will be cooled and absorbance of mixture will be measured at 535 nm against blank. Tocoferolwill be used as the reference antioxidant. The percent inhibition of hydroxyl radical generation will be calculated using the formula, [(C–T/C)] x 100, where C is absorbance of the control and T for the test samples.

5.7. In Vivo Dermatophytic Activity of Extracts In Mouse Model

From the results of the in vitro antioxidant and antidermatophytic assay extracts with significant activity will be considered for in vivo testing against (Swiss albino mouse model).

5.8 Experimental Animals and Ethical Considerations

Swiss albino rats of both sexes weighing 30-35 grams will be procured from the National Institute of Virology, Pune.

5.9 Acute Oral Toxicity Study

Acute oral toxicity study of the will be carried out using Swiss albino mice of both sexes. Lorke'smethod.

5.10 Selection and Preparation of Dose for Pharmacological Screening

The water ethanol, chloroform and hexane extracts of *Baliospermum* species will be suspended in 0.3 percent CMC (*Carboxy Methyl Cellulose*) solution to prepare two dose levels, 2000 and 400mg/kg body weight of the animals.

5.11 PHYSICAL PARAMETERS

5.11.1 Determination of liver weight

The animals will be sacrificed and the livers will be isolated and will behed with saline and weight determined using electronic weighing balances. The weight of the liver will be expressed in terms of its body weight (Ghosh et al., 2007).

5.11.2 Determination of Liver Volume

After the weight, livers will be released individually into a measuring cylinder with a fixed volume of distilled water or saline and the displaced volume will be recorded. (Ghosh et al., 2007)

5.12 BIOCHEMICAL PARAMETERS

The biochemical parameters will be estimated according to the standard procedure prescribed by the Manufacturing Instruction Manual given in the kit. (Coral Clinical Systems) using Semi Auto analyzer.

5.12.1 Estimation of Serum SGPT

5.12.2 Estimation or Serum SGOT

5.13.3 Estimation of Serum Alkaline Phosphatase (ALP)

5.13.4 Estimation of Serum Bilirubin

5.13.5 Estimation of Serum Cholesterol

5.13.6 Estimation of Serum Total Proteins

5.14 ANTIOXIDANT PARAMETERS

5.14.1 Estimation of Catalase

5.14.2 Superoxide dismutase

5.15 HISTOPATHOLOGICAL STUDIES

5.15.1 Processing of isolated liver

The animals will be sacrificed and the livers of each animal will be separated and cut into small pieces, preserved and fixed in 10% formalin for two days. The liver piece will be then will be held in running water for about 12 hours to remove the formalin and then dehydrated with isopropyl alcohol of increased potency (70%, 80% and 90%) for 12 hours. Dehydration is then performed using absolute alcohol with about three changes for 12 hours. The dehydration will be performed by discarding all traces of water. Further alcohol will be removed by using chloroform and chloroform removed by paraffin infiltration. The clearing will be completed by using chloroform with two changes for 15 to 20 minutes each. After paraffin infiltration the liver pieces will be placed to automatic tissue processing unit.

5.15.2 Embedding in paraffin vacuum

The hard paraffin will be melted and hot paraffin will be poured into L-shaped blocks. The pieces of liver will be then quickly dipped into the melted paraline and allowed to cool.

5.15.3 Sectioning

The blocks will be extruded using a microtome to obtain sections of 5 thicknesses. The sections will be taken on a micro slide on which egg albumin will be applied. Sections will be allowed to stay in the oven at 60°C for 1 hour. Paraffin melts and denatures egg albumin, which fixes the tissue to slide.

5.15.4. Staining

Eosin is an acid stain, so it causes pink spots on all the cell components that are in the basic form, i.e. the cytoplasm. Hematoxylin, a basic stain that causes a blue stain on all acidic cell components, namely: DNA in the nucleus.

5.16 STATISTICAL ANALYSIS

The results will be reported as mean ± standard error (SE), and the experiments will be replicated three times (n=3).

6. IMPORTANCE OF THE STUDY / SOCIETY APPLICATION:

A) International status:

One of the most important groups of fungi causing worldwide human and animal infections is dermatophytes. They are able to invade keratinized tissues, such as hair, skin, and nails to produce an infection dermatophytosis, which is commonly referred to as ringworm. The existing treatments for these infections are still limited to a few antifungal agents. However, clinical values of these agents have been limited due to having high toxicity and emergence of drug resistance in their antifungal activities. These factors emphasize the urgent need for the development of new effective treatment alternatives. Since the last decades, plant extracts and plant derived compounds, due to having fewer side effects, low cost, and high availability, have been valuable sources that are commonly used to treat a wide range of disease conditions including infectious diseases.**(Seyyed et.al.,2015)**

B.National status:

Over the past few years, it has been documented that dermatophyte infections haveincreased by many fold in India. Moreover, there is change in the disease presentation, severity, treatment responses, and relapse rate. Studies suggested that the emergence of *Trichophytonmentagrophytes* as the principle causative organism and high terbinafine resistance could be the cause of these changing patterns of the disease and responses. However, the causes may even be more diverse, from the irrational use of antifungal drugs to topical steroid usage and also to the poor socioeconomic status of the population. There are few studies from Karnataka, Sikkim, Delhi, and Himachal Pradesh, there are not much data available from eastern and Gangetic India where the disease is rampant.(Das et.al., 2020).

The antifungal drug market in India has grown by more than 100% in recent years following the epidemic-like situation of dermatophytoisis in the country. Almost every other pharmaceutical company has started marketing various antifungals drug. The quality and bioavailability of some the antifungal drugs by mushrooming companies are feared to be substandard which may add to this growing menace of difficult to treat dermatophytosis. Recurrent dermatophytosis is the topic of discussion in almost every social media groupsof Indian dermatologist. Although the problem of recalcitrant dermatophytosis is growing leaps and bounds in India, there is a lack of substantial published epidermiological, clinical, and laboratory research in this field. Many research projects, postgraduate thesis, and collaborative works are

underway to elucidate the factors that could have lead dermatophytosis to this crossroad in India.**(Dogra et.al.,2017).**

C. Regional status

The huge research gap has been came forth according to review of literature and this study lead some of the unnoticed medicinal values which have been practiced locals.

D. Significance of the study:

A major challenge in global health care is the need for novel, effective and affordable medicines to treat microbial infections. Therefore, the development of an alternative drug line of treatment for infectious diseases is of utmost importance. Plants have an amazing ability to produce a wide verity of secondary metabolites, like terpenoids, steroids, alkaloids, glycosides, saponins, flavonoids, tannins, quinines and coumarins. Some of these natural phytochemicals are highly resourceful in the treatment of bacterial and fungal infections**.(Radhakrishna and Kumari, 2019).**

Baliospermum species are orally used by people to cure stomach disorders and high fever respectively, but no work has been carried out on their antidermatophytic potential. If these plants showed better results against dermatophytes, it will be more beneficial to cure skin diseases.

7. PROPOSED WORK PLAN – With Tentative Chapter Scheme:

7.1. INTRODUCTION:

7.1.1. POTANTIA OF *BALIOSPERMUM* SPECIES:

A) Antidermatophytic Activity of *Baliospermum* Species.

B) Hepatoprotectivity Activity

C) Antioxident Activity

D) Cytotoxicity Assay

7.2. REVIEW OF LITERATURE:

7.3. MATERIAL METHODS.

a) Plant Material and Extract Preparation.

b) Cleaning and sterilization.

c) Test microorganisms and inoculum preparation.

d) Organic extracts preparation and their antifungal screening.

e) Qualitative and quantitative phytochemical analysis.

f) In Vitro Antioxidant Activity

g) In Vivo Dermatophytic Activity of Extracts In Mouse Model

h) Experimental Animals and Ethical Considerations

i) Histopathological Studies

j) Statistical Analysis

7.4. EXPRIMENTAL RESULTS.

7.5. DISCUSSION.

7.6. SUMMURY AND CONCLUSSION.

7.7. REFFERENCES.

8. Year-wise plan of work:-

1st Year:-

1. Review of Literature.
2. Collection of *Baliospermum* Sp.
3. Selection of dermatophytic fungi.
4. Referencing of work.

2nd Year:-

1. Preparation of powder.
2. Preparation of *Baliospermum* sp.extracts.
3. Assessment of antifungal activity against the dermatophytes.
4. Determination of Minimum Inhibitory Concentration (MIC)

5. *in-vitro* hepatoprotective, cytotoxicity and antioxidant activity of plant extracts.

3rdYear:-Completion of research work.

1. To study the *in-vivo* anti-dermatophytic activity of selected plant extracts by using suitable animal model.
2. Statistical study of all approaches.
3. Submission of thesis.

9. EXPECTED OUTCOME

Nowadays, people are focused on herbal medicine. They are blindly using medicinal plants to chronic diseases by listening to the Ram Dev Baba and reading the medicinal values of medicinal plants on multimedia. Every plant has hundreds of phytoconstituents which are harmful to the body and may cause hepatotoxicity and hence research should be carried out on the Oral Toxicity of these selected plant extracts.

It will be more beneficial to the society if such herbal remedy found to cure dermatophytic diseases and will be helpful to avoid hepatotoxicity if any by crud extracts and other side effects by allopathic treatment.

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